Original Research Article

DETERMINING THE RELATION OF TUMOR CHARACTERISTICS WITH IMMUNOHISTOCHEMICAL MARKERS IN WOMEN WITH BREAST CANCER

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A B S T R A C T

Background: Breast cancer is a significant global health concern, affecting millions of women each year. The molecular characterization of breast cancer has become crucial for tailoring personalized treatment strategies and predicting patient outcomes. Among the key biomarkers that guide treatment decisions, the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (Her2/neu) play pivotal roles in determining the disease behavior and response to therapy. Understanding HER-2 expressions and hormone receptors is essential for developing treatment strategies and making decisions regarding breast cancer. **Aims and Objectives:** The study was conducted to determine the prevalence of different subtypes based on the status of ER, PR, and Her2/neu receptors in this specific patient population and to Analyze the correlation between these molecular subtypes and clinicopathological characteristics, such as age, tumor size, grade, and lymph node status.

Materials and Methods: This is a Retrospective observational study conducted on 126 cases of breast carcinoma patients in Government General hospital, Kurnool. Slides were prepared from blocks containing cancer tissue, and immunohistochemical staining was done for ER PR & Her2neu expressions. Interpretation of expressions was done using Allred scoring system for ER, PR and the College of American pathologists guidelines for Her2neu expressions. Statistical analysis was performed to determine the statistical significance by applying chi-square test.

Results: The majority of the tumors were HER2/neu negative, positive for ER and PR. When it came to ER and PR positive tumors, most of them were Grade I. The current investigation established a correlation between age, tumor grade and ER and PR, but not with tumor size, lymph node status. There was a correlation between ER and PR expression, but not with HER2/neu.

Conclusion: Adjuvant hormonal therapy may result in a favorable outcome for cases that test positive for ER and PR. In order to provide prognostic information and therapeutic benefits, hormone receptor assessment is strongly advised for clinical management of breast cancer patients.

Keywords: HER2/neu, Hormone Receptor, Breast cancer, Lymphnode, Adjuvent.

INTRODUCTION

Breast cancer is a significant global health concern, affecting millions of women each year. Breast cancer incidence in females in India is 27% and the mortality associated with this cancer is 12.7% according to globacon 2022 statistics. Breast cancer incidence in world is 11.7% and the mortality associated with this cancer is 6.9% according to globacon 2022 statistics. The molecular characterization of breast cancer has

become crucial for tailoring personalized treatment strategies and predicting patient outcomes. Among the key biomarkers that guide treatment decisions, the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (Her2/neu) play pivotal roles in determining the disease behavior and response to therapy. This study aims to Determine the prevalence of different subtypes based on the status of ER, PR, and Her2/neu receptors in this specific patient population and to Analyze the correlation between these molecular subtypes and clinicopathological characteristics, such as age, tumor size, grade, and lymph node status in a cohort of 126 breast cancer patients attending Government General Hospital (GGH) in Kurnool.

The most prevalent malignant tumor and the primary cause of cancer-related fatalities in women is breast carcinoma. In industrialized nations, it is more prevalent.[1] Although there are many different kinds of breast cancer, the most prevalent histological kind is infiltrating ductal carcinoma.[2] When a tumor is localize, surgery is the cornerstone of breast cancer treatment. Chemotherapy and radio therapy are used as adjuvant treatment based on indications, in locally advanced disease. Chemotherapy is also used as neoadjuvant therapy and in metastatic disease as palliative therapy, and adjuvant hormonal therapy is used for cancers that express the progesterone and estrogen receptors.[3]

If detected in its early stages, breast cancer is treatable. The three classic morphological prognostic indicators are metastasis to axillary lymph nodes, tumor grade, and tumor size. Biological molecular prognostic variables are becoming increasingly important because many patients with early-stage breast cancer have microscopic metastases at the time of diagnosis.[4] Human epidermal growth factor receptor-2 (HER-2) and hormone receptors (ER and PR) are the most significant clinical indicators that are frequently utilized in the management stratification of breast cancer cases.[5] Understanding HER-2 expressions and hormone receptors is essential for developing treatment strategies and making decisions regarding breast cancer.[5] The treatment of breast cancer makes use of predictive and prognostic indicators. Prognostic factors are those that affect a patient's overall prognosis, like the likelihood of a recurrence following treatment. These elements have a part in choosing which patients will receive aspecified treatment.[6] Predictive factors assess the possibility that a certain treatment will be beneficial. There are prognostic and predictive variables for ER, PR, and HER2/neu.

Oestrogen Receptor: Throughout a woman's life, her breasts experience significant physiological changes that are actively mediated by estrogen. There are two forms of ER: ERα and ERβ.[7] One well-established predictor and prognostic factor for breast cancer is the receptor ERα. It's unclear how important ERβ is for prognosis.[7,8] While certain breast cancers only express ERβ, most ER-positive breast tumors have both ERα and ERβ subtypes. Different clinical behaviors and responses may result from this. It has been noted that ERβ expression decreases during breast carcinogenesis, in contrast to ERα.[9]

Progesterone Receptor: There are two varieties of PR: PR-A and PR-B. Estrogen function is modulated by progesterone.[10] It has been noted that ER-positive breast cancers without PR expression respond less well to hormone therapy than do PR-positive tumors. Furthermore, observed is the instability of the ER and PR phenotypes. These may alter as the illness progresses naturally or as a result of therapy.[11]

Human Epidermal growth factor receptor-2/neu: It belongs to the family of four closely related growth factor receptors, which also includes HER1, HER2, HER3, and HER4, also known as the human epidermal growth factor receptors. Breast cancer tumorigenesis and oncogenic transformation are mediated by HER2/neu amplification or over expression. When receptors are over expressed, signaling is inappropriately increased. The prognosis may worsen as a result of increased and uncontrollably proliferating cells, decreased apoptosis, increased motility of cancer cells, and angiogenesis.[12]

As there is a survival advantage for patients with hormone receptor positive status through treatment with adjuvant hormonal or chemotherapeutic regimens, determining ER, PR, and HER2/neu receptor status in breast cancer has become a common practice today. It is well known that, in contrast to patients with low to moderate ER positivity, strong ER-positive cases benefit from endocrine therapy alone. The presence of PR is independently linked to both overall and disease-free survival. Compared to patients with ER, PR-negative tumors, those with ER, PR-positive tumors have a better prognosis.[13]

Aims and Objectives

1. Determine the prevalence of different subtypes based on the status of ER, PR, and Her2/neu receptors in this specific patient population.
2. Analyze the correlation between these molecular subtypes and clinicopathological characteristics, such as age, tumor size, grade, and lymph node status.

MATERIAL AND METHODS

Study design: Retrospective observational study.
Study duration: August 2022 - October 2023 (Retrospective data period)
Sample size: 126 patients.

Slides were prepared from blocks containing cancer tissue, and immunohistochemical staining was done for ER PR & Her2neu expressions. Interpretation of expressions was done using Allred scoring system for ER, PR and the College of American pathologists guidelines for Her2neu expressions. Statistical analysis was performed to determine the statistical significance by applying chi-square test.

Inclusion Criteria

- Women aged between 20-70 years.

- ECOG Performance status 0-3
- Women who underwent treatment at GGH, KURNOOL
- Women who underwent Breast conservative surgery / Modified Radical mastectomy.

Exclusion Criteria
- Male breast cancers
- Patients who received treatment outside GGH, Kurnool
- Unresectable tumors.
- Data missing cases.
- Patients who lost follow up.
- Reccurent breast cancer.

RESULTS

Age

Patients were in the age group between 25-80 years, with mean age 50.69 years. The majority (35%)were in the age group between 41-50 years. Majority of ER positive cases were of age more than 50 years, Her2/neu positive were between 41-50. It was concluded that Her2/neu expression was statistically significant, indicating that Her2/neu positivity was seen at younger age than hormone receptor positive population.

Tumor size

The average tumor size was 4.4 cm. Majority of ER/PR/Her2neu positive tumors were of size between 2-5 cm. Correlation of expression of ER, PR, HER2neu compared to tumor size was statistically insignificant.

Grade

In our study, according to Nottingham Modified Bloom-Richardson system score, majority of tumors were in Grade I (47%) followed by Grade II (41.2%) and then Grade III (11.1%). Majority of ER/PR/Her2neu positive tumors were of Grade I. It was concluded that ER, PR expression compared to tumor grade was statistically significant and Her2/neu was not significant.

Lymph node status

All cases were evaluated for axillary lymph nodes metastasis and found that 59 patients had lymph nodes metastasis. Out of 70 ER positive, 27 had positive axillary lymph nodes, whereas out of 62 PR positive ,26 had axillary lymph nodes metastasis. Out of 63 Her2/neu positive cases, 34 had positive axillary lymph node metastasis. It was concluded that correlation of expression of ER was statistically significant and PR, HER2/neu was statistically insignificant.

Receptor Positivity

Seventy tumors were ER positive and 56 were ER negative. ER positive tumors showed weak, moderate to strong nuclear positivity in >1% of tumor cells.

Sixty-two tumors were PR positive and 65 were PR negative.PR positive cases showed weak, moderate to strong nuclear positivity in >1% of tumor cells.

Out of 126 cases,58 were ER and PR positive, 56 were negative and 12 showed different expressions of ER and PR. On statistical analysis, it was observed that k= 0.413; asymptomatic standard error= 0.081; P= 0.000 and using kappa as measure of agreement, it was concluded that expressions of ER and PR agree significantly to each other.

Sixty-three were HER2/neu positive and 63 were HER2/neu negative. A total of 15 cases were both ER and HER2/neu negative. Out of 126 cases, 16 cases were PR and HER2/neu positive ,17 were both PR and HER2/neu negative. On statistical analysis using kappa as measure of agreement, it is concluded that expressions of ER/PR and HER2/neu do not agree with each other.

Table 1: Age distribution

Age	ER			PR			HER2/neu		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
<40	14	9	23	12	11	23	8	15	23
41-50	20	24	44	24	20	44	28	16	44
51-60	24	19	43	17	26	43	26	17	43
>60	12	4	16	9	7	16	1	15	16
Total	70	56	126	62	64	126	63	63	126

	Chi square	Degrees of freedom	P value
ER	5.690	3	0.128
PR	2.000	3	0.572
Her	18.547	3	0.000

Table 2: Tumor Size

Tumor size	ER			PR			HER2/neu		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
< 2	8	2	10	8	2	10	2	8	10
2-5	36	25	61	26	35	61	36	25	61
> 5	26	29	55	28	27	55	25	30	55
Total	70	56	126	62	64	126	63	63	126
	Chi square		Degrees of freedom		P value				
ER	4.502		3		0.212				
PR	5.972		3		0.113				
Her	6.224		3		0.101				

Table 3: Grade

Grade	ER			PR			HER2/neu		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
I	44	16	60	37	23	60	33	27	60
II	21	31	52	17	35	52	22	30	52
III	5	9	14	8	6	14	8	6	14
Total	70	56	126	62	64	126	63	63	126

	Chi square	Degrees of freedom	P value
ER	14.759	2	0.001
PR	9.754	2	0.008
Her	2.116	2	0.347

Table 4: Lymphnode status

Lymph node status	ER			PR			HER2/neu		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Positive	27	32	59	26	33	59	34	25	59
Negative	43	24	67	36	31	67	29	38	67
Total	70	56	126	62	64	126	63	63	126

	Chi square	Degrees of freedom	P value
ER	4.309	1	0.038
PR	1.172	1	0.279
Her	2.582	1	0.108

Table 5: Receptor Positivity

ER	Her2/neu +	Her2/neu-	Total	PR	Her2/neu +	Her2/neu-	Total
ER Positive	22	48	70	PR Positive	16	46	62
ER Negative	41	15	56	PR Negative	47	17	65
Total	63	63	126	Total	63	63	126

	K	ASE	P value
ER	0.413	0.081	0.000
PR	-0.476	0.078	0.000

DISCUSSION

Breast cancer is the most common cancer in women, making up 25% of all cases globally and the primary cause of cancer-related deaths in women. In developed nations, it is more prevalent. The type of cancer, the extent of the illness, and the patient's age all affect the outcome of breast cancer. In the developed world, the five-year survival rate is high, at 90% in the US and 80% in England.[15] The rates of survival in developing nations are low. This is explained by the absence of screening programs that are effective and the general lack of knowledge about the symptoms and signs of breast lumps, which can result in advanced disease with a larger tumor and nodal involvement at presentation.[16]

Data from India indicate that among females, the most common site of cancer is the Breast. The mainstay of breast cancer treatment is surgery when a tumor is localized, followed by chemotherapy (when indicated), radiotherapy and for ER and PR positive tumors, adjuvant hormonal therapy. ER, PR, and HER2/neu are immunohistochemical markers of prognosis as well as predictors of response to therapy. At present also, determining ER, PR, and Her2/neu receptor status in breast cancer have become common practice as there is a survival advantage for patients with hormones receptor positive status by treatment with adjuvant hormonal or chemotherapeutic regimens.

AGE

Women 50 years of age and older receive a diagnosis in over two thirds of cases of breast cancer; most of these cases occur in developed nations.[17] In developing nations compared to developed nations, twice as many cases of breast cancer are diagnosed in women between the ages of 15 and 49.Following guidelines for routine screening is linked to a lower death rate from breast cancer in nations where mammography is accessible and reasonably priced.

Patients were in the age group between 25-80 years, with mean age 50.69 years is similar to the study conducted by Senegal et al,[5] and Kaul et al,[18] The majority (35%)were in the age group between 41-50 years. Majority of ER positive cases were of age more than 50 years as seen in study conducted by Alzaman et al,[19] Her2/neu positive were between < 50 years similar to Alzaman et al,[19] observations.

Tumor Size

The average tumor size was 4.4 cm. Majority of ER/PR/Her2neu positive tumors were of size between 2-5 cm. There was no significant correlation observed between tumor size and ER, PR, HER2/neu expression as compared to the Almasri and Hamad.[21] study.

Tumor Grade

In our study, according to Nottingham Modified Bloom-Richardson system score, majority of tumors were in Grade I (47%) followed by Grade II (41.2%) and then Grade III (11.1%). Majority of ER/PR/Her2neu positive tumors were of Grade I. There was seen significant correlation between tumor grade with ER (P=0.001) and PR(P=0.008). The study done by Dodiya et al,[20] showed similiar results. No association was seen between tumor grade and HER2/neu expression(P=0.347) similar to the study done by Dodiya et al.[20]

Axillary Lymph Node Status

All cases were evaluated for axillary lymph nodes metastasis and found that 59 patients had lymph node metastasis. Out of 70 ER positive, 27 had positive axillary lymph nodes, whereas out of 62 PR positive,26 had axillary lymph node metastasis. Out of 63 Her2/neu positive cases, 34 had positive axillary lymph node metastasis. Significant correlation was observed with ER (P= 0.038). No significant correlation was observed between axillary lymph node status with PR(P=0.279) and HER2/neu(P=0.108) expression, similar to studies conducted by Azizun-Niza et al.[22]

RECEPTOR POSITIVITY

In the present study, ER positivity was 55%, closely matched the results of the study conducted by Idirisinghe et al,[23] and PR positivity was 49 %, closely matched the results of the study conducted by Engstrøm et al,[24] HER2/neu positivity was only 50 % was much higher as compared to other studies. The possible explanation for this is due to variations in different populations. In addition, HER2/neu assay results are influenced by multiple biologic, technical and performance factors. Since many aspects of HER2/neu assays have not been standardized, the effects of these disparate influences could not be isolated. ER and PR correlated with each other (P = 0.000), whereas expression of HER2/neu was inversely related to ER (P = 0.000) and PR expression (P = 0.000). Similar results were found in studies conducted by Maha et al.[25]

CONCLUSION

The range of age for breast carcinoma in our study was 25 to 80 years, with a mean age of 50.69 years. The age group of 41-50 years accounted for a greater number of cases. The majority of the tumors were HER2/neu negative and positive for ER and PR. Most of the ER and PR positive tumors, were Grade I. The current investigation established a correlation between age, tumor grade, ER and PR, but not with tumor size and lymph node status. There was a correlation between ER and PR expression, but not with HER2/neu. Adjuvant hormonal therapy results in a favorable outcome for cases that tested positive for ER and PR.

In order to provide prognostic information and therapeutic benefits, assessment of hormone receptor is strongly recommended for clinical management of breast cancer patients.

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